[redacted]

March 17, 2021 **Final**

R-21-0001 GENETIC CONSTRUCTION REPORT
ANALYST: Khoa Nguyen, Ph.D.
SUBMITTOR: [redacted]

INTRODUCTION

The submitter has engineered [redacted] bacterial strains they identify as [redacted], [redacted], [redacted], and [redacted] (Table 1). These are the subject strains of TERA R-21-0001. An important note is that these are all subject strains of a single TERA case number (R-21-0001), as opposed to individual case numbers. To assist in keeping things clear, the following table includes case sub-numbers.

[redacted]

The proposed generic name for the subject microorganism is "*Bacilli* strain engineered to affect nitrogen production" (MCAN sec. 2.2). The confidential use is [redacted]" The [redacted] subject strains are intended for experimental environmental releases to evaluate their ability to enhance nitrogen acquisition by plants. The genetic modifications were [redacted].

The subject strains were developed using [redacted] and [redacted]. These strains are the result of [redacted]) of the [redacted]) containing an [redacted] and the [redacted] gene from [redacted] This was followed with transforming with [redacted], containing the genes of interest flanked by [redacted]). These [redacted] are recognized via the [redacted] by the [redacted], allowing for [redacted] of the genes of interest into the [redacted] genome. Both [redacted] and are not maintained in any of the final [redacted] subject strains.

During the development of the subject strains, various endogenous [redacted] genes [redacted]. This resulted in either a [redacted] of the gene or a [redacted] in the subject strains. The endogenous genes/loci include [redacted]), [redacted]), [redacted]), [redacted]), [redacted]), [redacted] *and* [redacted] The functions of the disrupted genes are listed in Table 3.

[redacted]

An overview schematic of how the subject strain was constructed is provided in Figure 1 and a detailed schematic is provided in the accompanying file (R-21-0001 GCR Schematic FINAL.pptx).

A summary of microbiological, genetic, and biochemical details related to the intergeneric genes in the subject strain is provided in Table 2 and corresponding details are provided in section II. The addition of intrageneric genes and the deletion of endogenous genes are addressed in sections II.D and II.E, respectively.

[redacted]

[redacted]

I. INTENDED USE

The [redacted] subject strains are intended for experimental environmental releases to evaluate their ability to enhance nitrogen acquisition by plants (specifically in corn plants; TERA sec 4.0). The genetic modifications were [redacted] (TERA sec 1.1).

II. DESCRIPTION OF THE GENETIC MODIFICATIONS

A. The Parental and recipient strains

The parental strain is described as [redacted] This parental strain [redacted] was then transformed with a [redacted], [redacted] carrying the [redacted] gene involved in [redacted] This intermediate strain is considered the recipient strain for all eight subject strains. Since the submitter did not name this recipient strain, it will be referred to as [redacted] for this assessment.

B. Overview

Per the TERA, the parental [redacted] strain [redacted] was transformed with the [redacted], [redacted] and the [redacted] from [redacted]. This intermediate, recipient strain [redacted]) was then transformed with [redacted], which contains the [redacted] [redacted]. At this stage, the [redacted]) was allowed to [redacted] the corresponding DNA, flanked by the [redacted], from the [redacted] to the [redacted] genome in a [redacted] step between [redacted]. Lastly, the resulting strains were selected on [redacted] at a [redacted], allowing for [redacted], yielding the final subject strains.

C. Addition of intergeneric genes

While the [redacted] gene is the only one that originated outside the [redacted] genus, other [redacted] genes used are also considered intergeneric due to DNA modifications to [redacted] sites used during the [redacted]. These include: [redacted] there were no DNA modifications, therefor it is considered intrageneric.

[redacted]

This gene is located on all [redacted] as part of the [redacted] and is integrated into each of the [redacted] subject strains. The [redacted] gene used in this TERA encodes [redacted]. While cloned from the [redacted] plasmid from [redacted], this gene is also found in a variety of bacteria ([redacted]). This [redacted] gene, including the promoter [redacted] and terminator [redacted]) were PCR amplified from a [redacted]

[redacted]

[redacted] (TERA Table 3) with primer tails that affixed the [redacted] and [redacted] as well as [redacted] to create a [redacted] TERA Att. 2).

The [redacted] by [redacted] thereby generating [redacted]). [redacted]

Per the TERA, this [redacted] gene [redacted].

Figure 2. Illustration of [redacted]

[redacted]

As noted above, although the following genes are from the genus [redacted], they are considered intergeneric due to genetic modifications made to their DNA sequences to [redacted] for [redacted].

[redacted]
This is in subject strains [redacted].

[redacted]

For subject strain [redacted], [redacted]

[redacted]

[redacted]),
This is in subject strain [redacted].
[redacted]

[redacted])
This is in subject strain [redacted].
[redacted]

[redacted]
Present in subject strain [redacted]
This codes for a [redacted] from [redacted], consisting of [redacted] and [redacted] Individual genes are described below:

- [redacted]

The submitter hypothesized that [redacted]

[redacted]

[redacted]

This is in subject strain [redacted].
This gene codes for a [redacted] from [redacted]).
[redacted]

[redacted]
This is in subject strain [redacted].
This gene codes for a [redacted]
[redacted]

[redacted]

[redacted]

[redacted]

D. Addition of Intrageneric genes:

[redacted]
This is in subject strain [redacted].

[redacted]

[redacted]

[redacted]

E. Deletion/Disruption of Endogenous Genes

Per the TERA, the [redacted]

The predicted integration site/disrupted gene is shown in the table below for each subject strains, along with their known functions.

[redacted]

F. Step-by-step modifications

The remaining details are well and concisely described in the TERA (Sec 2.2.3). An overview of the subject strains and their genotypes is in Appendix 1 of this report. TERA Attachment 2 contains information on the plasmids used to construct the subject strains. Sequence data are also provided in TERA Attachments 4-6.

A summary schematic of the genetic construction of the subject strains is provided in Figure 1.

[redacted]

III. INSERTED SEQUENCE VERIFICATION

[redacted] from each subject strain were [redacted] (TERA sec 2.2.4). Sequencing was performed using [redacted] which is an adaptation of the [redacted]. [redacted] produced between [redacted] reads per sample. The maximum read length was between [redacted]

The submitter confirmed that the [redacted] are intact and that the desired [redacted] containing the [redacted] marker and the genes of interest were inserted without any deviation from the synthesized sequence for all eight subject strains (TERA sec 2.2.3).

On the issue of genetic stability of the inserted material, the submitter noted that all modifications were integrated directly into chromosomal DNA. No loss of the genetic material has been observed by the submitter over numerous instances and many generations of culturing strains for research and development activities (TERA sec 2.2.5).

[redacted]

While various [redacted] were elements of the [redacted] used to engineer the subject strains, the only one that remained in the final subject strains is the [redacted] gene, encoding a [redacted] from [redacted] providing [redacted]. [redacted], providing [redacted] and [redacted] encoding the class [redacted] providing [redacted] like [redacted], were part of the [redacted] which does not remain in any of the final subject strains.

[redacted]

IV. REFERENCES

1. [redacted]

[redacted]

Appendix A

[redacted]